MAJOR ARTICLE

New Treatment Approach in Indian Visceral Leishmaniasis: Single-Dose Liposomal Amphotericin B Followed by Short-Course Oral Miltefosine

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Background. In Bihar, India, home to nearly one-half of the world's burden of visceral leishmaniasis, drug resistance has ended the usefulness of pentavalent antimony, which is the traditional first-line treatment. Although monotherapy with other agents is available, the use of 2 drugs with different modes of action might increase efficacy, shorten treatment duration, enhance compliance, and/or reduce the risk of parasite resistance. To test the feasibility of a new approach to combination therapy in visceral leishmaniasis (also known a kala-azar), we treated Indian patients with a single infusion of liposomal amphotericin B (L-AmB), followed 1 day later by short-course oral miltefosine.

Methods. We used a randomized, noncomparative, group-sequential, triangular design and assigned 181 subjects to treatment with 5 mg/kg of L-AmB alone (group A; 45 subjects), 5 mg/kg of L-AmB followed by miltefosine for 10 days (group B; 46 subjects) or 14 days (group C; 45 subjects), or 3.75 mg/kg of L-AmB followed by miltefosine for 14 days (group D; 45 subjects). When it became apparent that all regimens were effective, 45 additional, nonrandomized patients were assigned to receive 5 mg/kg of L-AmB followed by miltefosine for 7 days (group E).

Results. Each regimen was satisfactorily tolerated, and all 226 subjects showed initial apparent cure responses. Nine months after treatment, final cure rates were similar: group A, 91% (95% confidence interval [CI], 78%–97%]; group B, 98% (95% CI, 87%–100%); group C, 96% (95% CI, 84%–99%]; group D, 96% (95% CI, 84%–99%); and group E, 98% (95% CI, 87%–100%).

Conclusions. These results suggest that treatment with single-dose L-AmB followed by 7–14 days of miltefosine is active against Indian kala-azar. This short-course, sequential regimen warrants additional testing in India and in those regions of endemicity where visceral leishmaniasis may be more difficult to treat.

Trial registration. ClinicalTrials.gov identifier: NCT00370825.

Efforts to improve the treatment of visceral leishmaniasis (also known as kala-azar) and develop new therapeutic approaches have moved steadily forward in the past decade. This progress has been driven, in part, by the emergence of large-scale resistance to conventional pentavalent antimony (Sb) treatment in Bihar, India, which accounts for ~90% of India's (and ~45% of the world's) cases of kala-azar [1, 2]. Recent therapeutic advances in Indian kala-azar include demonstration of the efficacy of short-course treatment using the lipid formulations of amphotericin B [3], identification of miltefosine as the first effective oral agent [4], and rediscovery of paromomycin [5].

In parallel with the development of new single-drug regimens, there is also growing interest in resurrecting the notion of combination therapy to treat visceral leishmaniasis, as practiced, for example, in the treat-

Received 26 April 2008; accepted 19 June 2008; electronically published 9 September 2008.

The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy, or views of the World Health Organization.

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Clinical Infectious Diseases 2008; 47:1000–6

1058-4838/2008/4708-0003$15.00
DOI: 10.1086/591972

ment of tuberculosis, HIV infection, and malaria. Such an approach, in the form of Sb plus aminosidine (paromomycin), was tested initially in Kenya, India, and Sudan in the 1990s and enhanced overall efficacy and/or reduced treatment duration [6–8]. Combination Sb-paromomycin therapy is currently in use in Sudan [9]. Other potential advantages of 2-drug chemotherapy in the treatment of kala-azar include: (1) less toxicity (as a result of lower drug doses and/or shorter treatment courses); (2) convenience, better compliance, and lower costs, resulting from less lengthy treatment; and (3) possibly reduced likelihood of developing resistance to either agent. In view of the fate of Sb in Bihar [1], there is also understandable concern about how to best preserve the efficacy of new single-agent treatments that are now being deployed, especially in regions of endemicity, such as India, where transmission is anthroponotic [2,10].

Using 2 self-administered oral drugs would be ideal in kala-azar; however, only 1 drug, miltefosine (Impavido; Zentaris), is currently available [2, 4]. Therefore, in considering what might constitute optimal combination therapy in Bihar, where Sb is not an option, we focused this pilot trial on testing the particularly active parenteral agent liposomal amphotericin B (L-AmB), which is marketed under the brand name AmBisome (Gilead Sciences), with short-course miltefosine. In devising the test regimens, we took advantage of 2 prior findings in the same Indian population: (1) a single infusion of L-AmB by itself at 7.5 or 5 mg/kg can induce cure rates of 90%–91% [11, 12], and (2) although miltefosine is ordinarily given for 28 days [4], limited data suggest reasonable activity (89% cure rate) with a 14-day course [13]. Thus, if used in combination (in this case, sequentially), reducing the duration of miltefosine treatment and/or lowering the dose of L-AmB both appeared to be feasible.

PATIENTS AND METHODS

This study, carried out from August 2006 through March 2007 at the Kala-Azar Medical Research Center in Muzaffarpur, Bihar, India, was approved by the Center's ethical committee and registered with ClinicalTrials.gov (identifier: NCT00370825).

Trial design. This trial was designed as a randomized, parallel-arm, noncomparative, open-label study using a group-sequential (triangular test) method to reach—with the minimum number of subjects—an early decision as to which regimens should be selected for additional testing. Of note, in a classic single-stage comparative trial design with a type 1 error α of 5%, a power $1 - \beta$ of 95%, and the null hypothesis of 90% efficacy, a sample size of 580 patients per arm would be needed to reach the significance level for a regimen with 95% efficacy.

The study was designed to have a 5% type 1 error and 95% power ($\alpha = .05$ and ß $= .05$), considering a failure rate of <10% to indicate adequate efficacy (the minimum detectable failure rate at the ß $= .05$ level) and a failure rate ⩾25% to indicate insufficient efficacy. The boundaries of the test were calculated for H0 ($p = p0$) and Ha ($p < pa$) with $p0 = 0.25$ and $pa = 0.10$. Based on simulations, we expected the sample path to cross the H0 rejection line with an average sample size of 40 patients and the H0 non-rejection line with an average sample size between 20 and 25 patients. When, after enrolling 45–46 patients per arm, all treatments appeared to be equally and highly effective, an additional 45 consecutive patients were enrolled and nonrandomly assigned to a fifth regimen.

Eligibility and entry and exclusion criteria. Patients ⩾12 years of age were eligible for the study if they had symptoms and signs of kala-azar (e.g., fever, weight loss, and splenomegaly) and parasites demonstrated by microscopic examination of splenic aspirate smear [4, 5]. Pregnant or breast-feeding women and individuals who were seropositive for HIV or who had a serious concurrent infection, such as tuberculosis or bacterial pneumonia, were excluded. Exclusion criteria also included granulocyte count <1000 cells/μL, hemoglobin level <3.5 g/dL or platelet count <40,000 platelets/μL, hepatic transaminase levels or total bilirubin >3 times the upper limit of normal, serum creatinine level >2.0 mg/dL, and prothrombin time >5 sec above control. If they were randomized to receive miltefosine, women of child-bearing age with negative pregnancy test results were counseled about the potential teratogenic effects of miltefosine and were offered contraception in the form of a depot preparation of progesterone. All such women enrolled in this study gave consent and received the injection.

Trial procedures and treatments. Subjects completed baseline testing (including standard biochemistry and hematology profiles, urinalysis, examination of chest radiographs, electrocardiogram, anti-HIV antibody testing by ELISA, malaria smear, and pregnancy testing) and provided written informed consent. An independent statistician generated a randomization schedule by use of a computer-based procedure; assumptions were a maximum number of 60 patients enrolled per arm (240 total patients) and 15 randomization blocks with a size of 15 patients each. Sealed randomization envelopes were prepared, and treatment was begun within 72 h after diagnosis by splenic aspirate. All subjects received L-AmB once on day 1 as a 2-h intravenous infusion [11, 12]; antipyretic pretreatment was not used. Miltefosine was started on day 2 and was administered at 100 mg per day (50 mg twice-daily with meals) [4]. Drugs were generously donated by Gilead Sciences (L-AmB) and Zentaris (miltefosine); neither manufacturer had a role in the planning, conduct, or analysis of the study or in the preparation and publication of this article.

Initially, 181 subjects were randomly assigned according to a group-sequential (triangular) design to receive either 5 mg/kg of L-AmB administered once (group A), 5 mg/kg of L-AmB

administered once plus miltefosine for either 10 days (group B) or 14 days (group C), or 3.75 mg/kg of L-AmB administered once plus miltefosine for 14 days (group D). After initial posttreatment assessments on day 16 showed apparent cure responses in all 181 subjects, the protocol was amended to include a fifth group of 45 subjects (group E) with use of the same inclusion and exclusion criteria. These nonrandomized subjects received 5 mg/kg of L-AmB administered once followed by miltefosine for 7 days.

Patients were kept in our inpatient unit throughout treatment and were examined daily. Complete blood counts and serum creatinine and hepatic transaminase levels were obtained on day 8 and day 16 or when warranted clinically; limited resources precluded additional laboratory testing. On day 8, miltefosine treatment was to be withheld if the creatinine level doubled from baseline and exceeded 2.0 mg/dL or if any value exceeded 2.5 mg/dL; miltefosine treatment was not to be restarted until the level decreased to 1.4 mg/dL, the upper limit of normal. Adverse events were also classified according to the Common Toxicity Criteria as mild (grade 1), moderate (grade 2), severe (grade 3), or very severe (grade 4) [4, 5]. Treatment was to be discontinued and subjects removed from the study if adverse events of Common Toxicity Criteria grade $\geqslant 3$ occurred, with the exception of increases in liver transaminase levels. Such increases may occur during miltefosine therapy, but they do not preclude continuing treatment [4].

Splenic aspirate for apparent cure evaluation was repeated on day 16. For groups A, B, C, D, and E, day 16 represented 15, 5, 1, 1, and 8 days, respectively, since the last treatment dose. Parasite density score for pretreatment and posttreatment splenic aspirate smears was graded by microscopic examination in a blinded fashion with use of a conventional logarithmic scale of 0 (indicating no parasites per 1000 oil-immersion fields) to +6 (indicating >100 amastigotes per field) [4, 5]. Designation of apparent cure on day 16 required clinical improvement, a reduction in spleen size, and a splenic aspirate score of 0 (i.e., apparent parasitological cure) [4, 5]. Definitive cure, assessed after 9 months, required being healthy with no signs or symptoms of relapse [4, 5]. All subjects were given 400 rupees (US $9) to offset the costs of travel to the 9-month visit. Patients who did not return on time were contacted in person. No subject was lost to follow-up.

Statistical analysis. For continuous variables, data are expressed as mean value ($\pm$SE). Frequencies are given as percentages with 95% CIs. Pretreatment (baseline) characteristics were compared between groups to determine any imbalances by using a 1-way analysis of variance. Continuous data were assessed for normality using the Kolmogorov-Smirnov test. If statistically significant, data were log-transformed and analyzed using the Student's *t* test, if normally distributed; the Mann-Whitney *U* test was used for paired comparisons. Homogeneity of variance was assessed with the Bartlett test. The Welch adjusted analysis of variance was performed if variances were unequal. Dichotomous variables were analyzed using the χ^2 test.

In the design of the trial, cure rate at day 16 was considered to be the primary end point and, for the purposes of this study, was used as a surrogate for cure rate at month 9. The day 16 cure rate was analyzed for every 5 subjects in each noncomparative arm using the triangular test.

Secondary parameters were also considered for comparisons between day 0 and day 16. Normally distributed differences between baseline and day 16 were analyzed with a paired Student's *t* test. Non–normally distributed data, assessed by the Kolmogorov-Smirnov test, were analyzed by the Wilcoxon signed rank-sum test. All tests were 2-tailed except the triangular test. A P value of $<.05$ was considered to be statistically significant. Data were analyzed using SAS, version 9.1.3 (SAS Institute).

RESULTS

Figure 1 summarizes the trial profile and table 1 shows baseline clinical and laboratory results. Twenty-two subjects (10%) had received prior treatment, 19 with Sb, in equal proportions across all groups. At baseline, patients in the 5 groups were similar except for higher WBC counts ($P = .04$) and platelet counts ($P < .001$) in groups C, D, and E than in groups A and B. Clinically severe kala-azar (i.e., spleen size >8 cm and hemoglobin level <7.0 g/dL [14]) was present in 1–4 patients in each of groups A–D and in none of the patients in group E.

All 181 subjects in groups A–D completed assigned treatments with no drug interruptions, and on day 16, 100% had parasite-free splenic aspirate smears and fulfilled the criteria for apparent cure (tables 1 and 2). Because of identical initial responses, 45 additional subjects (group E) were enrolled and treated with 5 mg/kg of L-AmB administered once followed by an abbreviated (7-day) course of miltefosine. All group E patients completed treatment, and all 45 patients were also apparently cured at the day 16 assessment. Additional posttreatment results indicated significant decreases in spleen size and increases in weight, hemoglobin levels, and WBC and platelet counts in each patient group (comparing values at day 16 vs. baseline values; table 1).

Higher temperature, rigors, or both responses developed during L-AmB infusion in 9–13 subjects (20%–29%) in each of the 5 groups. These anticipated infusion-related reactions [11, 12, 15] were transient, and single-dose L-AmB treatment was considered to be well-tolerated. Posttreatment (day 16) results for each group also showed no meaningful change in serum creatinine levels nor, with the exception of group C, in hepatic transaminase levels (table 1).

Adverse events in individual patients are shown in table 3, in which day 8 corresponds to 1 week after treatment with L-

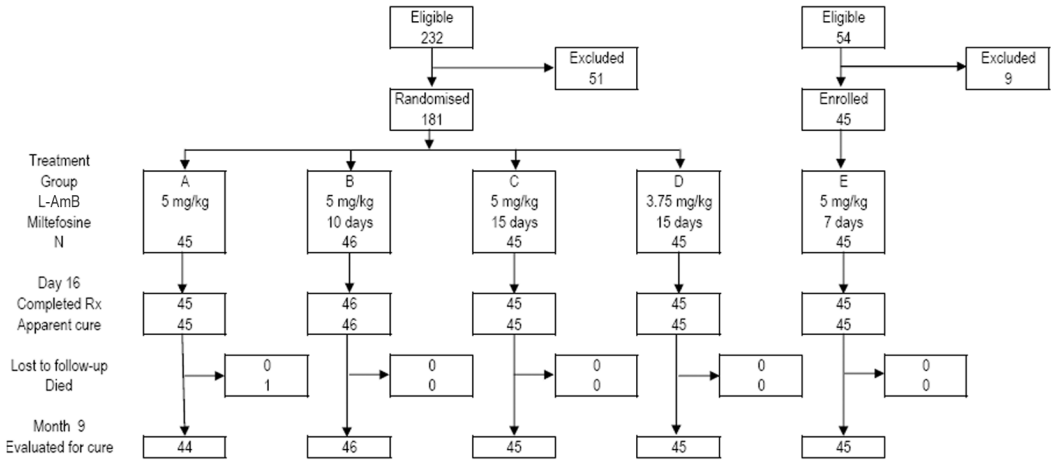

Figure 1. Trial profile for a new treatment approach in Indian visceral leishmaniasis using single-dose liposomal amphotericin B (L-AmB) followed by short-course oral miltefosine.

AmB alone (group A), end of treatment in group E, three-quarters through treatment in group B, and halfway through treatment for groups C and D. Similarly, day 16 represents the following posttreatment intervals: group A, 15 days; group B, 6 days; group E, 8 days; and in groups C and D, the day treatment ended. Vomiting and diarrhea during the first week of therapy and increases in serum creatinine and serum glutamic pyruvic transaminase levels were anticipated in miltefosine-treated patients [4]; however, these reactions also occurred in several individuals who received L-AmB alone. In general, adverse events were mild (grade 1) or moderate (grade 2) and, in most instances, resolved or improved to grade 1 by day 16. However, 3 group C subjects developed Common Toxicity Criteria grade 3 (severe) hepatotoxicity (which resolved during continued miltefosine treatment), and a fourth patient experienced an asymptomatic grade 4 reaction (very severe) with a serum glutamic pyruvic transaminase level that reached 3425 IU/mL. When retested 2 weeks after treatment, this subject's serum glutamic pyruvic transaminase value had returned to normal.

During the 9-month posttreatment period, 1 group A patient died during a flood 5 months after treatment (table 2). In addition, 9 patients developed symptomatic, parasitologically confirmed relapses (7 during months 1–6 and 2 during months 7–9). All 216 remaining patients were asymptomatic and appeared to be healthy at month 9 and were judged to have shown a definitive cure response. Thus, overall cure rates, by intention-to-treat analysis, were as follows: group A, 91% (95% CI, 78%–97%); group B, 98% (95% CI, 87%–100%); group C, 96% (95% CI, 84%–99%); group D, 96% (95% CI, 84%–99%); and group E, 98% (95% CI, 87%–100%). The 9 patients who experienced relapse were retreated successfully using full-dose L-AmB therapy (15 mg/kg total dose, administered at a dosage of 3 mg/kg/day for 5 days) [15].

DISCUSSION

In India, kala-azar is conventionally treated with a single agent. Although Sb has now been lost as a viable treatment option in Bihar because of the development of drug resistance [1, 15], alternative therapies have emerged and are effective, although they are not without certain drawbacks [2, 15]. For example, intravenous treatment with amphotericin B deoxycholate usually requires 30 days in the hospital and may be difficult to tolerate [14]; a 5-day course of intravenous L-AmB (e.g., total dose of 15 mg/kg [15, 16]) is expensive despite its newly discounted price (US $20 per 50-mg vial); a 28-day course of oral miltefosine remains fairly expensive, even at the discounted price offered to the World Health Organization (US $84 for a patient weighing >25 kg), although drug cost is largely offset by savings from outpatient self-administration [17]; and, although inexpensive, paromomycin requires 21 days of daily intramuscular injections [5].

Therefore, in the preceding context and versus monotherapy, the use of 2 active antileishmanial drugs with distinct mechanisms of action may have potential advantages, such as enhanced overall efficacy, the ability to maintain high-level cure rates when using either lower drug doses or shorter treatment periods, less drug-related toxicity, and the possibility of limiting the emergence of resistance to either agent. The possibility of emerging drug resistance has already been raised for miltefosine [18].

The results of this pilot study indicate the feasibility of a particular approach to combination treatment in Indian kala-azar that takes advantage of the effectiveness of single-dose L-

Table 1. Baseline and posttreatment clinical and laboratory data for patients treated for Indian visceral leishmaniasis using single-dose liposomal amphotericin B (L-AmB) followed by short-course oral miltefosine.

	Group A		Group B		Group C		Group D		Group E	
Variable	Day 0	Day 16	Day 0	Day 16	Day 0	Day 16	Day 0	Day 16	Day 0	Day 16
No. of patients enrolled	45	…	46	…	45	…	45	…	45	…
Age, mean years (±SEM)	27 ± 2.0	…	32 ± 2.0	…	29 ± 2.0	…	25 ± 2.0	…	28 ± 2.0	…
Male sex, % of patients	58	…	61	…	64	…	71	…	51	…
Prior therapy, no. of patients	2	…	6	…	4	…	5	…	5	…
Duration of illness, mean days (±SEM)	45 ± 3	…	43 ± 4	…	42 ± 6	…	45 ± 5	…	43 ± 4	…
Splenic aspirate score, mean value (±SEM)	1.9 ± 0.17	0	1.5 ± 0.1	0	1.5 ± 0.12	0	1.8 ± 0.16	0	1.4 ± 0.11	0
Weight, mean kg (±SEM)	36.9 ± 1.6	37.6 ± 1.7[a]	40.8 ± 1.7	41.6 ± 1.6[a]	37.3 ± 1.5	38 ± 1.5[a]	36.2 ± 1.7	37 ± 1.7[a]	39.7 ± 1.4	40 ± 1.4[a]
Spleen size, mean cm (±SEM)	4.2 ± 0.6	1.4 ± 0.3[a]	3.7 ± 0.3	0.8 ± 0.2[a]	3.3 ± 0.4	0.5 ± 0.1[a]	3.9 ± 0.6	0.73 ± 0.2[a]	3.4 ± 0.3	0.6 ± 0.2[a]
Hemoglobin level, mean g/dL (±SEM)	7.8 ± 0.3	9.3 ± 0.3[a]	8 ± 0.3	9.6 ± 0.2[a]	7.5 ± 0.3	9 ± 0.3[b]	7.8 ± 0.3	9.7 ± 0.2[a]	8.2 ± 0.3	9.6 ± 0.3[a]
WBC count, mean cells/μL × 10^3 (±SEM)	2.7 ± 0.2	5.3 ± 0.4[a]	2.8 ± 0.2	6.0 ± 0.3[a]	3.2 ± 0.3	8.1 ± 1.9[a]	3.1 ± 0.2	6.3 ± 0.3[a]	3.3 ± 0.3	6.2 ± 0.3[a]
Platelet count, mean platelets/μL × 10^3 (±SEM)	107 ± 9	205 ± 12[a]	105 ± 7	207 ± 11[a]	140 ± 17	225 ± 15[a]	110 ± 8	233 ± 12[a]	132 ± 16	234 ± 12[a]
Creatinine level, mean mg/dL (±SEM)	0.8 ± .03	0.7 ± .03[a]	0.8 ± 0.03	0.8 ± .03	0.8 ± 0.02	0.8 ± .03	0.8 ± 0.04	0.7 ± .03	0.7 ± 0.03	0.7 ± .02
SGPT level, mean IU/mL (±SEM)	38 ± 4	38 ± 3	46 ± 5	36 ± 3	39 ± 5	110 ± 75	36 ± 5	38 ± 3[c]	36 ± 3	33 ± 3[d]

NOTE. Group A received 5 mg/kg of L-AmB administered once. Group B received 5 mg/kg of L-AmB administered once plus miltefosine for 10 days. Group C received 5 mg/kg of L-AmB administered once plus miltefosine for 14 days. Group D received 3.75 mg/kg of L-AmB administered once plus miltefosine for 14 days. Group E received 5 mg/kg of L-AmB administered once plus miltefosine for 7 days. All subjects received a single infusion of the indicated dose of L-AmB on day 1. In groups B–E, miltefosine was started on day 2 and given for the indicated periods. SGPT, serum glutamic pyruvic transaminase.

[a] $P < .001$.
[b] $P = .002$.
[c] $P = .007$.
[d] $P = .032$.

Table 2. Responses to treatment for patients treated for Indian visceral leishmaniasis using single-dose liposomal amphotericin B (L-AmB) followed by short-course oral miltefosine.

Variable	Group A	Group B	Group C	Group D	Group E
L-AmB dose, mg/kg	5	5	5	3.75	5
Duration of miltefosine treatment, days	0	10	14	14	7
Enrolled	45	46	45	45	45
Completed treatment	45	46	45	45	45
Apparent cure on day 16	45	46	45	45	45
Relapse	3	1	2	2	1
Death	1	0	0	0	0
Lost to follow-up	0	0	0	0	0
Definitive cure at 9 months[a]	41	45	43	43	44
Percentage of patients with definitive cure at 9 months[a] (95% CI)	91 (78–97)	98 (87–100)	96 (84–99)	96 (84–99)	98 (87–100)

NOTE. Data are no. of patients, unless otherwise indicated. Group A received 5 mg/kg of L-AmB administered once. Group B received 5 mg/kg of L-AmB administered once plus miltefosine for 10 days. Group C received 5 mg/kg of L-AmB administered once plus miltefosine for 14 days. Group D received 3.75 mg/kg of L-AmB administered once plus miltefosine for 14 days. Group E received 5 mg/kg of L-AmB administered once plus miltefosine for 7 days. All subjects received a single infusion of the indicated dose of L-AmB on day 1.

[a] Patients who experienced relapse (n = 9) or died (n = 1) by month 9 were designated as having experienced treatment failure.

AmB [11, 12] and the efficacy and convenience of oral miltefosine [4, 17]. Our findings suggest that the use of these 2 agents in tandem may permit a new short-course approach, potentially allowing the duration of miltefosine therapy and the amount of L-AmB administered to be reduced. Both effects may help mitigate the cost of using 2 expensive drugs. (The estimated drug cost in India for a 40-kg patient in group E, for example, would be US $101.) Our results also suggest that sequential treatment with these 2 agents is satisfactorily tolerated and, as judged by initial and long-term responses, does not interfere in any meaningful fashion with either parasitological or clinical efficacy. As with any miltefosine-containing

Table 3. Adverse clinical and laboratory reactions among patients treated for Indian visceral leishmaniasis using single-dose liposomal amphotericin B (L-AmB) followed by short-course oral miltefosine.

	Group A (*n* = 45)		Group B (*n* = 46)		Group C (*n* = 45)		Group D (*n* = 45)		Group E (*n* = 45)	
Reaction	Day 8	Day 16	Day 8	Day 16	Day 8	Day 16	Day 8	Day 16	Day 8	Day 16
Vomiting										
Grade 1	0	0	0	0	0	0	0	0	0	0
Grade 2	5	0	9	0	5	0	6	0	1	0
Diarrhea										
Grade 1	3	0	6	0	4	0	4	0	2	0
Grade 2	0	0	2	0	3	0	1	0	0	0
Creatinine level										
Grade 1	0	3	12	5	4	4	4	3	5	0
Grade 2	0	0	2	0	0	0	0	0	2	0
SGPT level										
Grade 1	13	10	12	9	15	8	15	10	18	10
Grade 2	1	0	2	2	0	0	3	1	1	0
Grade 3	0	0	0	0	3	0	0	0	0	0
Grade 4	0	0	0	0	0	1	0	0	0	0

NOTE. Group A received 5 mg/kg of L-AmB administered once. Group B received 5 mg/kg of L-AmB administered once plus miltefosine for 10 days. Group C received 5 mg/kg of L-AmB administered once plus miltefosine for 14 days. Group D received 3.75 mg/kg of L-AmB administered once plus miltefosine for 14 days. Group E received 5 mg/kg of L-AmB administered once plus miltefosine for 7 days. All subjects received a single infusion of the indicated dose of L-AmB on day 1. In groups B–E, miltefosine was started on day 2 and given for the indicated periods. For clinical reactions, results (Common Toxicity Criteria grade) indicate the number of patients in whom reactions developed during days 1–8 (day 8) or days 9–16 (day 16). Laboratory results show the numbers of patients in whom the indicated reactions were present on either day 8 or 16. SGPT, serum glutamic pyruvic transaminase.

regimen, this form of treatment is contraindicated in pregnant women; in addition, women of child-bearing age need to maintain effective contraception during and for 2 months after treatment [4].

It is important to note that this trial was designed to identify suitable options for combination treatment deserving further study and to discard less active regimens. The study was randomized to avoid selection biases when enrolling patients, although a fifth group (group E) was added when it became clear that all 4 of the regimens that were initially tested were apparently effective. This trial was noncomparative and was not designed (nor was it of sufficient size) to address other relevant questions. These include: (1) whether the addition of miltefosine meaningfully improves upon the ~90% cure rate of treatment with single-dose L-AmB alone [11, 12]; (2) whether any of the tested combination regimens are as effective as giving miltefosine alone for 28 days (which has a 94% cure rate for hospitalized subjects and protocol-adherent outpatients [4, 17] and an 84% cure rate for all outpatients [17]); and (3) what is the proper dose of L-AmB and proper duration of miltefosine treatment to use in tandem? Future studies will be needed to address such questions.

Nevertheless, although they are preliminary, our results do suggest the possibilities of using L-AmB at <5 mg/kg (e.g., at 3.75 mg/kg, as in group D) as well as oral therapy in courses as short as 7–10 days (as in groups E and B). If this particular combination approach to kala-azar in Bihar is supported by additional trials aimed at inducing a cure rate of >95% [14, 19], 1 infusion of L-AmB (in most instances, administered in an outpatient setting) followed by a brief self-administered course of miltefosine would be a regimen with clear-cut clinical appeal.

Acknowledgments

We acknowledge both Gilead Sciences and Zentaris for generously donating AmBisome and Impavido, respectively.

Financial support. Sitaram Memorial Trust, the Varanasi Physicians Research and Education Foundation, and Drugs for Neglected Diseases Institute.

Potential conflicts of interest. S.S. has received support for clinical trials and travel funds to attend scientific meetings from Asta Medica/Zentaris and NeXstar/Gilead Sciences. All other authors: no conflicts.

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